

device to produce a more laminar flow. In addition, directing the cell aerosol into matrix materials as the matrix is forming in the space between the target or mandrel and the source(s) of molecules being electroprocessed produces the effect of cushioning the cells. While not wanting to be bound by the following statement, it is believed that the cells will be trapped in the storm of filaments or other bodies produced by electrospinning or electroprocessing and pulled onto the mandrel. This situation may be less traumatic to the cells than directly spraying the cells onto a solid surface.

In one embodiment, the cells are added either before or at the same time as the materials or compounds that form electroprocessed materials are brought together. In this way, the cells are suspended throughout the three-dimensional matrix. In embodiments in which the electroprocessed material comprises fibrin formed by combining thrombin and fibrinogen, the cells are typically included in the mixture that contains the fibrinogen (whether it is plasma or purified fibrinogen). Whenever materials comprise two or more separate materials that combine to form a different material (such as fibrinogen and thrombin) bringing the materials together immediately prior to insertion into a mold, or immediately prior to the streaming step in the electrospinning process helps result in a good distribution of cells in suspension in the resulting extracellular matrix.

Cells can be added as the filaments are produced in the space between the target and polymer source. This is accomplished by dripping the cells onto the target, dripping the cells into the matrix as it forms, aerosoling the cells into the matrix or onto the target or electrospraying the cells into the matrix as it condenses and forms near or on the grounded target. In another embodiment, cells are sprayed or dribbled into a forming electroprocessed material or matrix and thereby trapped as the electroprocessed material crosses the air gap between the source solutions and target.

An alternative method to deliver cells to an electroprocessed material involves electroaerosol delivery of the cells. Cells can be deposited by electrostatic spraying at, for example, 8kV directly onto standard polystyrene culture dishes, suggesting that electrostatic cell spraying is a viable approach. Cardiac fibroblasts in phosphate buffered saline (PBS) have been electroaerosoled up to a 20 Kv potential difference. In another example, Schwann cells (rat) were plated on a PS petri dish by conventional methods after one day. Schwann cells were also electrosprayed onto a PS petri dish with a

metal ground plate behind the dish at 10kV after one day. Both samples grew to almost confluence after one week. The electroaerosol approach provides some distinct advantages. First, the shear forces produced during the delivery phase (i.e. the production of the aerosol) appear to be much less traumatic to the cells.

5 Second, the direction of the aerosol can be controlled with a high degree of fidelity. In essence the cell aerosol can be painted onto the surface of interest. This allows the cell to be targeted to specific sites. In electroaerosol delivery, cells are suspended in an appropriate media (e.g. culture media, physiological salts, etc.) and charged to a voltage, and directed towards a grounded target. This

10 process is very similar to that used in electroprocessing, particularly electrospinning. The produces a fine mist of cells trapped within the droplets as they are produced and directed at the grounded target.

Cells can be delivered using aerosol and electroaerosol techniques onto an electroprocessed material that is forming by an electroprocessing technique. The

15 electroaerosol of cells can be delivered in parallel (i.e. alongside) the electroprocessing material or from a separate site. The cells can be delivered to the storm of filaments or particles produced within the air gap in the electrodeposition process or directed at the target. The cells and electroprocessed material also can be delivered in an alternating sequence to the target, i.e.

20 electrodeposit the material, aerosol the cells, electrodeposit the material, aerosol the cells. This allows for the discrete layering of the construct in separate layers. Furthermore, a vapor source can be provided that directs water onto the mandrel of target used to collect the cells. Providing this moisture improves cell viability by keeping the cells from dehydrating during processing. Cells can be added to

25 the electroprocessed material at any time or from any orientation in any aerosol strategy. Again the advantage of the process in general is that collagen, for example, collects in a dried state on the target mandrel. Accordingly, although some solvents for collagen may be toxic, they are lost from the system before the filaments collect on the target.

30 Cells can also be trapped within a carrier prior to producing an aerosol. For example, cells can be encapsulated within a material like alginate. The encapsulated cells are physically protected from shear and trauma during processing. Cells delivered in this form to the electroprocessed material will have higher viability when sprayed or electrostatically seeded.

35 An electroaerosol or otherwise electroprocessed material can also be

delivered directly to an *in situ* site. For example, an electroprocessed material can be produced directly onto a skin wound, with or without substances such as molecules or cells. Additional cells or materials can then be aerosolized onto or into the wound site. Other surgical sites can also be amenable the delivery of materials using various electrodeposition techniques or combinations thereof of these methods.

In other embodiments, substances can be applied to the electroprocessed material after formation, for example by soaking the electroprocessed material in the substance or by spraying the substance onto the electroprocessed material.

Persons skilled in the art will recognize that more than one method for combining the substances with electroprocessed materials can be used in a single embodiment or application. Combining methods can be especially useful in embodiments involving release of more than one compound or compounds intended to have complex release kinetics, although such combinations are not limited to those embodiments.

Magnetically and electrically active materials can be electroprocessed, including, for example, preparing conducting polymer fibers produced by electrospinning. In addition, conducting polymers can be prepared in-situ in the matrix by, for example, incorporation of a monomer (e.g., pyrrole) followed by treatment with polymerization initiator and oxidant (e.g.,  $\text{FeCl}_3$ ). Finally, conducting polymers can be grown in the material after electroprocessing by using a matrix-coated conductor as the anode for electrochemical synthesis of, for example, polypyrrole or polyaniline. Compounds that can form electroprocessed materials can be added to an aqueous solution of pyrrole or aniline to create a conducting polymer at the anode with the entrapped electroprocessed material-forming compounds, which can then be treated with other compounds to allow formation of the material to occur.

#### *Patterns of electroprocessed materials and substance distribution*

Many embodiments of the present invention involve means for manipulating the pattern or distribution of electroprocessed materials and/or substances within an electroprocessed material. For example, an electroprocessing target can also be specifically charged or grounded along a preselected pattern so that electroprocessed materials streamed toward the target are directed into specific directions or distributions on the target or on a substrate.